

November 21, 1947.

1... Streak out presumed + and - leuved from 58-161 on EMB- β gal. etc

β gal galac. galac+ ϕ H

- a) ++ ++ no growth W52
- b) - ++ no growth W53. (58-161 purified re β gal +)

Note: this is failure to ferment, not growth inhibition.

2... leuved from Y10.

segregation of $\beta\phi$, lac.

27a.

W52 x Y53.

November 21, 1947.

Stocks which are $\beta\phi gal^+ lac^+$ and $\beta\phi gal^- lac^-$ are available. If there are three alleles:

lac^+ , lac^+ and lac^- , only the parents should be recoverable. If there are two loci, the type $lac^+ \beta\phi^-$ should be found in this cross. It can be controlled by testing the cross $lac^+ \beta\phi^- \times lac^- \beta\phi^-$ which should not segregate for $\beta\phi$. For additional segregating characters, Hef may be used.

A21 broc cultures into 25 ml 1/25 YP broth. incubate overnight at 37°.

A22. Transfer 5 ml ea. to new 25 ml YP.

incubate 9 AM -

wash, etc., mix in T(0)

and T(B₁) plates.

A. W52 x Y53

B. W53 x Y53

A24. Suspend in 1 ml H₂O and streak out on lac EMB to obtain single colonies of lac^+ and lac^- segregants.

November 24, 1947.

A WS2 x Y53 *lac* + ϕ + x *lac* - ϕ -
 B. WS3 x Y53. *lac* + ϕ - x *lac* - ϕ -.
 $\beta\phi$ + $\beta\phi$ -

Streak out prototypes on
 EMB *lac* agar and separate
lac - from *lac* + in pure form.
 Test them on ϕ gal.

A (0) 8: -

A (B). 20 *lac* - } all appear to score $\beta\phi$ + on mass streaks.
 7 *lac* +. } Streak out β + and β - on a $\beta\phi$ plate.

B (B). 16 *lac* -
 1 *lac* +

incl. parentals.

	$\beta\phi$	<i>lac</i>
WS3	+	+
Y53	+	- !
<i>lac</i> - seg.	+	-
" "	+	-
<i>lac</i> + seg.	+	+
" "	+	+

B (0) 1 +
 2 -

It is possible that ϕ gal- is not adept on $\beta\phi$ electride but that lactose splits the glycoside!
 N. O.

Assumption that Y53 is $\beta\phi$ + *lac* - is mistaken.
 all parentals must be retested.

Complementary Genotypes.

Nov. 25, 1947.

487

410

Plan. Cross $B-M-T+L+B_1+Lac-V_1^R \times B+M+T-L-B_1-Lac+V_1^S$

and recover B_1-Lac- segregants. Plate these colonies into BMTL lactose agar to suppress the parental and major recombinant type. The only types which could survive are B_1+Lac+ which includes the complementary genotype ~~$B+M+T-L-B_1+Lac+V_1^S$~~ $B-M-T-L-B_1+Lac+V_1^S$ and also possible recessions of $B+M+T-L-B_1-Lac+V_1^S$ in αB_1- . This procedure affords at least some chance, however, of recovering the complementary type by selective means. 20 colonies plated.

#11, 13, 14, 15, 17. are $Lac-$ (i.e. 5/20). Throw out other plates. Strains there out.

II.		III.
1	T	1 11-1
2	T	2 13-1
3	T	3 4-1
4	T	4 15-1
5	T	5 15-2
6	separate colonies	6 15-3
7	separate colonies.	7 15-4
8	T	8 15-5
9	T	9 17-1
10	T	10 17-2
11	S.C. 1 colony.	11 17-3
12	T	12 18-1
13	1 colony.	13 18-2
14	S.C. 1 colony. Pick.	14 18-3
15	S.C. >10 colonies. Pick 1-5.	15 18-4
16	T	16 18-5.
17	3 colonies. Pick 1-3.	
18	>10 colonies. Pick 1-5.	
19	T	
20	T	

BM	TLB ₁	BMTL ₁
-	+	+
+	-	+
+	-	+
-	+	+
+	+	+
+	+	+
+	+	+
+	-	+
+	-	+
+	-	+
-	+	+
-	+	+
-	+	+
+	+	+
+	+	+

$B-M-$ probably are recessions of Lac ; TLB_1+ maybe B_1- recessions of the TLB_1 parent. Use maltose instead which does not seem to allow recessions!

Pick out and test single $Lac+$ colonies for nutrition and phage. compare 3 segregants.

No complements found.

Comparison of various grades of sugars for EMB test.

November 20, 1947.

Malt+ Mal- Lact+ Lac-

EMB +1%:

Lactose c.p. [+++.] - allst+.

Lactose U.S.P. +++ ± all-

Maltose, c.p. (Paragon) +++ - allst+

Maltose, c.p. (E+A) +++ - allst+

Maltose, purified (Mills) +++ - allst+

Maltose, technical (E+A) +++ ± allst±

Larger colonies than c.p. lactose. Probably minimal amounts of monosaccharide.

36hr. readings.

+++ denotes good sized colonies with deep, uniform purple-black coloration; and a green metallic sheen. ± is faint pink coloration, suitable for scoring.

- denotes pale or translucent colonies. alls refuses development of blue coloration.

Technical grade sugars, therefore, seem to be suitable for preparation of EMB plates. Hereafter unless otherwise specified, EMB plates for mutant detection will be made up from Lactose U.S.P. (milk sugar) Mallinckrodt and Maltose (Malt Sugar) Technical, E+A.

Concn, program, approx. follow:

Maltose (c.p.) (Tech) (U.S.P.) .03 .002

Lactose .002 .001

Adaptation Expts: Prelim.

Nov. 18, 1947.

Cells grown in lactose, β - ϕ galactoside + glucose are sedimented and washed. Resuspension ca 10^9-10^{10} cells/ml. Cells diluted to comparable concentrations. Add 1ml cells to 1ml 4% sugar + .01 ml M/5 phosphate buffer pH 7.0. Add 0.3 ml BCP, 15% putube as indicator.

Made up 11.15 AM.

acid production ma + ... +++ seal.

		11:30	11:45	1:30	A 19.
Str 1	glucose	-	-	-	+++
	lactose	-	-	-	+++
	β - ϕ gal	-	-	-	-
Lac 1	lactose	++	+++	✓	++
	β - ϕ gal	-	-	-	-
10 ϕ 1	lactose	-	-	-	+++ ++
	β - ϕ gal.	-	-	-	-

Urease in coli.

Nov. 20, 1947.

Purposive media with peptone 1% agar 1.5%, Phenolphthalein 0.1% ± glucose 0.2%, ± urea 2%.

After autoclaving, phenolphthalein turned slightly. This subsequently disappeared.

	A21	A22	A24
-	growth, no color	✓	turning pink
Glucose	" "	✓	✓
Urea	Growth inhibited	growth, no color	✓
Urea + Glucose.	Growth inhibited.	" "	✓

This does not seem to be a satisfactory method for demonstrating urease.

Nov. 26, 1947.

Mix up media containing 1/2% NaFormate, 1% peptone, 1/2% agar and various indicators, ± glucose .3%

1. EMB. glucose a) 24h. 36 do.
 glucose + formate b) all colonies. light lavender.
2. Phenolphthalein .01%
 formate a) diffuse opacity; growth inhibited somewhat.
 - b) no reaction, good growth.
3. Bromocresol purple. Add AcOH to medium until turned acid.
 glucose a))
 glucose + formate b)) no growth
 formate c)) (pH?)

EMB seems to be the most suitable, using glucose + formate.

Methyl Green sulfate - lactose:

Lac + colonies green, diffusing into agar
 Lac - colonies translucent light blue.
 n. satist because of diffusion

EMB + sugar 1%: does, streaked - out 58/61.

gentiobiose -
 β methyl glucoside -
 α phenyl glucoside + uniformly.

Colony formation on synthetic agar.

Nov. 25, 1947

T(m) agar + various concentrations of sugars. Old BMTLB₁.

Lactose:

	24h. 58-161.	36h.	24h. 487.	36h.
.1%	absent.	2 mm.	microscopic pinpoint; papillae.	
.05%	small, definite.	1-2 mm.	microscopic pinpoint (1.1 mm)	
.01%	pinpoint.	<u>1 mm.</u>	no visible colonies; none.	

.1% is a satisfactory level of carbon supplementation.

Later, 487 shows continually forming papillae on all plates.

On .1%, 487 forms distinct colonies certain proportions of which contain reversions. .01% is also suitable.

November 25, 1947.

Cross W52 x W-1 in O, B, agar.

B-M-T+L+B,+lac+B ϕ +Mal+ x B+M+T-L-B,-B ϕ -Lac-Mal-.

Carry up very slowly and in small numbers. Segregants
not used in view of 27b.

~~Use for mal segregation:~~

Nov. 26, 1947.

Streak out 58-161 on EMG agar: .3% $\text{NH}_4\text{H}_2\text{PO}_4$, 1.2% galactose
A 76.

A Definite colony demorphosis as previously described. : ●
about 1:1 S R.

B. Streaks out components and mixture on galactose EMG.
A 76.
W-28 + W-29.

Reversion? of C-2 mutants.

36.

Nov. 29, 1947.

Plate 24hr. YP cultures into agar supplemented as indicated.
10⁸ per plate

Y138: T(0). No colonies.

Y138: Arginine : 1 colony?

Lysine : No colonies.

Arg + Lys. No colonies. Not turbid!!!

Y142. T(0). >30 colonies.

+ val + val. "

+ arginine + val + val. >100 cols. Only sl. turbid.

+ arg. turbid.

Y138 + Y142 ... O >30 cols.
A. turbid. colonies form.

Check the requirements of these strains!!

Check C₂ mutants.

11/29/47.

	Y	T(0)	T:	T:	T:	F:	
1.	114.	0:-	iso-	val-	i+v. +	++ ³⁶	48 hrs. OK!
2.	117	0:-	³⁶ arg. +++			++ ³⁶	adapted.
3.	120	0:-	✓ val +	++ ³⁶		OK!	OK! Try crossing with 138, 139, or make mutants from this strain.
4.	121	0:-	³⁶ ++ cyst +++				adpts.
5.	132	0:-	arg.-	gly-	arg.-	no growth ^{36h.} ✓	AS Both A+AG +++ Recheck Reg.
6.	133	0:±	arg ±	³⁶ lys ±	³⁶ arg ±	+++	adpts.
7.	134	0:	arg	thr	arg		
8.	137	0:	arg	trp	arg		
9.	138	0:-	arg-	leuc-	arg leu	+++	OK. all OK.
10.	139	0:-	arg-	hist-	arg hi	+++	OK. ^{T(0)} OK others adapted.
11.	142	0:-	³⁶ i+v -	³⁶ arg +++ ⁺⁺⁺	³⁶ i+v + arg. ⁺⁺⁺		all ⁺⁺⁺ . Requires arginine only! adpts on minimal too!

First readings at 24h., 2d at 36, 3d at 48. Inc. at 37°.

Y142 is very adaptable. Y138 + Y139 are fairly stable, especially Y138. do. Y120. and Y114.

Utilization of starches.

Dec 2, 1947

- .05% in T(m) (BM) and 1% in EMB.
- A Amylose (Clinton - from K.P.L.)
 - B Amylopectin (do.)
 - C Waxy starch, soluble, from Brink.
 - D. Glucose.

P 11. Continued, slow utilization of amylopectin noted. to "++" compared to +++ for glucose.
v. slight utilization of ~~W~~ W_x noted.

P 16. Continued increase in turbidity. density = ca. ~~1.01~~ 1.01% glucose

P 24 Utilization apparently complete. Rate measurements were exceedingly crude. Waxy starch was not utilized to nearly the extent that amylopectin was. This should be repeated for confirmation. Save flasks of amylopectin culture.

Exp. terminated this date.

Jan. 7, 1948. Compare mould from B with V55 inoculum on T(m) BM + falouring.

	α	β	John ^{#17} color
A p. .05%	\pm	$+$	light red-rod.
Amylose .05%	$-$	\pm	blue
Waxy starch.	$++$	$+++$	No color
	\pm	\pm	blue blue
	$+$	$+$	Light red
	$-$	$-$	As dark red.

see 86.

all starch utilizations are correlated then. Possibility of adaptation, rather than cumulative utilization not excluded. Compare inulin on EMB!

Synthetic EMB Medium.

Nov. 29, 1947.

Medium, per l.	/100ml.
Na Succ. $\cdot 6H_2O$. 5	1
Lactose 10	2
$(NH_4)_2SO_4$ 5	1
NaCl 5	1
Mg SO_4 1	.2

EMB; Agar

(Phosphate is in EMB mixture).

OK! *Empress* K-12 +++
B₁-lac- -- (E B, added).

Dec 1, 1947.

W-1 x W-53.

T-L-B₁-Lac-Mal- $\beta\phi$ + x B-M-Lac+Mal+ $\beta\phi$ -

a) T(10) plates.

	Mal+	M-
Lac+	2	15
L-	2	44

b) T(15) plates.

	Mal+	Mal-
Lac+	1	10
Lac-	2	47

Total:

Lac+	3	25	/ 123.
Lac-	4	91.	

in %

	M+	M-
L+	2.4	19.7
L-	3.1	71.6

Total Lac+ = 22.7%

	M+	-
L+	2.4	20.3
-	3.2	74.0

Lac+ = 22.7
Lac- = 77.2

Mal+ = 7.6
Mal- = 94.3.

∴ Mal is v. closely linked to B-M. Evidently not to B₁ in view of homogeneity of distribution.

probably between B and Lac. This leads to an excess of the triple type, M+L+. Check on each purported example here of M+. Check ✓. Scores correct.

Dec. 3, 1947.

From numerous plates is 41, streaked down on maltose agar + count, pick out M+ for lac characterization. (T.M. plates).

M+	M-
1	38
0	47
1	50
1	27
3	36
4	43
3	31

$$\frac{15 \cdot 272}{288} = 13.5$$

$$Mal+ = \frac{13}{285} = 4.6\%$$

Test all Malt + on lactose:

M+	lac+	lac-
	10	5

Summary of ~~lac~~ ^{lac} distribution among ~~Mal+~~ ^{Mal+}:

+	-
3	4
10	5
13	9
	22

Total distribution:

M- ^{lac+}	M- ^{lac-}	M+
272	116	15
388	7	22
94.5%	74.1%	5.5%
	20.4%	2.2%
	3.3%	

400.

From same plates as 41, segregate lac+ and lac- and streak an isolated colony on β gal agar, EM10.
at 24 hours:

	lac+	lac-
β gal +	20	36 + 1
β gal -	0	170
	20	37

The parents were compared by streaking from YP broth and, unfortunately are not comparable. Neither W-1 nor W-53 was readable at 24h.

~~Isolate all segregants to small agar slants.~~

Parents are also both β gal+ and cannot be distinguished. A modifier may enhance β gal-ase in ~~W-52~~ W-52.

To summarize, all available lac+ are β gal+

The "lac-" of Y53+der., Y87+der., ^{W30}W40 and W42 are β gal+; The "lac-" of W35;36, W43, W45, W48, W49

Maltose synergism.

41d.

Cell suspension stored 2 days in H₂O at room temperature was plated on T(0) and T(B₁) as well as EM(B).

On EM(B), None of ¹⁷¹⁻⁴⁹ was Maltose +.

On 3 comparable plates only 2 possible Malt+.

On T(0). None of 139, streaked to Maltose, was lact+.

(Check for B₁ interaction again.)

On EM(B), lact synergism was:

Plate rather crowded.

+	-	
40	66	
16	37	
56	103	/159

Some colonies were noticed to be sectored!, as if complementary or supplementary types were present.

In this sample, therefore, only 2 / >600 was Malt+. Compare with above!

12/147

lac- $\beta\phi$ -Lac+ $\beta\phi$ +

W45

x Y10.

T(0)

T(B₁). Strains to synthesize Lac(β ₁). →

Few or no Lac- noted on EMS-lactose crossing plates [Spiegelman phenomenon?].

Strains lac- and Lac+ to $\beta\phi$ gal

$\beta\phi$ +	lac -	Lac +
	0	40
$\beta\phi$ -	12	0

Lac+ Lac-

68 9

58 3

126 12. / 138.

lac- 8.6%!

This is a much lower proportion of lac- than without found.

[Check for alleles with Y53.]

Suggests identity of $\beta\phi$ and lac loci.

cf.

Maltose Segregation:

43.

A. Y40 x W-1

Lac⁺ Mal⁺

Lac⁻ Mal⁻

~~B. W-20 x Y64.~~

~~Lac⁺ Mal⁻~~

~~x Lac⁻ Mal⁺~~

all plates too crowded.

On EMS nearly all Mal⁻. < 1:100 Mal⁺. These can be picked out more readily than in the reverse cross. However, the plates are too crowded to be very useful. Use T(10) etc. plates to confirm ratios.

Y120 x Y138.

41.

12/4/47.

do 43 for cells.

(D) base into minimal only (plates).

Y120 10^{-7} colonies

Y138 No colonies - two plates

Y120 + Y138. As above

~~Add to YP flasks for further incubation.~~

Y120 is too revertible for sex tests

12/4/47.

Inoc. Y120 into YP. Inoculate 3ml. suspension in
quartz flask. PS.

A6. Inoc. 10^{-7} dilution into T (Val) plates (detection).

A8. Layer 1% Y.G., 1% NZFase, 1% Hg on plates.

15 plates. Sample counts:

58
72
71
54
70
54
60
7 | 419 = 60 ~~5~~ average

(3) small colonies recovered.

-1 Not mutant, though inhibited by isoleucine

-2 Contaminant

(3) see ff.

Test Jan. 6. 1948: Valine +

1.	-	3do.	-
2.	NA		-
3.	EA		-
4.	N+EA		-
5.	MC (GB1)		±
6.	NZCase		+
7.	Y.G.		+++

Acc. 5 ~~7~~ 6 cells neg. on vials.

December 4, 1947.

Y45 x Y53. On T(B₁) and EMS Lac(B₂)

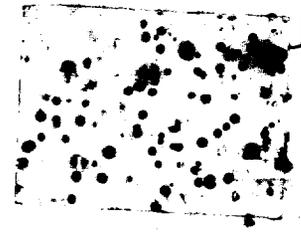
On EM(B).

Ca. 1:6 Lac+ : Lac- !!

[This suggests faulty identification of W45 as Lac-
 1) May be Lac+
 2) May be Lac₂ - Lac₁ +

Yields seem to be higher on EM(B). Come up with varying lag.

~~16~~ + -
 ca. 16. 40



From T(B₁).

-	+	
25	4	
19	7	
31	8	
22	8	
<hr/>		
97	27	/124



W45 x Y53.

Repeat 46.

Check parents: Both -. W45 allelic gene.
 lact+ present in cross! [of 41 isolates from T(0), 8+
 33 -

Struck out from T(B₁) on lac EMB agar to purify. also, 29- 4+
 62- : 12+ / 74
 ca 5:1

On EMS Lac, most plates too heavy.

3 Thiamin,

+	-	
3	11	
6	11	
6	12	
3	3	
<hr/>		
18.	37	/ 55

The EMS procedure seems to be hard for lact+ compared to T(0) plating. It should possibly be improved.

i Thiamin.

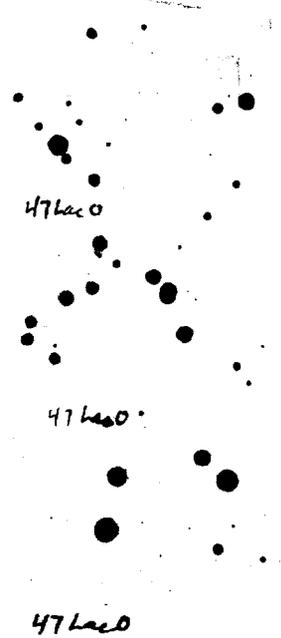
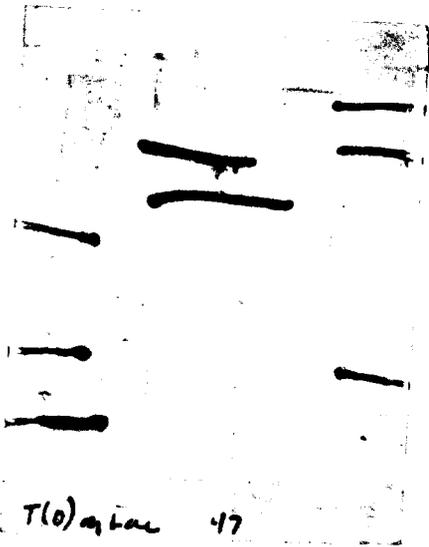
9	20	
14	31	
12	35	
34	56	
<hr/>		
69	142	/ 211

Read from paper impression slips.
 Give same average ratio, however
 of 67% lac+, whereas the random isolations give 80% Lac-.

χ^2 for difference is, approximately, at the 5% level.

8	33	41
14	28	
<hr/>		

 $\chi^2 = ca. \frac{36}{14} + \frac{36}{28} = 24.5.$



Maltose Segregation

W-21 x Y64

β -M-T+L+B+Lact+Mal-V₁^S x β +M+T-L B₁ Lac-Mal+V₁^R

On EMB, nearly all Mal+.
≪ 1:100 Mal-

Cf. 43, reverse cross where
Mal+ is very rare.



Dec. 8, 1947.

a). Raffinose 3%, Melibiose 1% + Salicin 1% EMBS. Streaked 58-161.
Dec. 7.

R - to ± A9.

Sal. - ~~±~~

Meli ++ Colicam therefore split glucose α-galactoside but not sucrose α-galactoside!

b). Same sugars, .05% in T(m) + DM. 48 hours reading.

A9.
R ±
Sal ±
Meli +++
Glucose ++)

↳ Streak to Salicin EMBS and inoculate second tube of T(m) + Salicin. + and - colonies seen. Selection for Sal+ has therefore been successful. Sal+ is W-55

Test 453, W-45 m melibiose : both +++.

E-M-S- Modification.

EMS: old formula, + : , strains K-12. Read at 48-72 hours.
(succinate)

		Growth	Color.
K Glucarate	.1%	± +	- ±
	.2%	++	+
	.5%	++	++
	1%	++	+++
Glucose	.05%	+++	-
	.1%		±
	.2%		+
	.5%		+++

Glucose .05% Maybe useful. Try with Na formate equimolar, or perhaps with K-saccharate.